β-Amyloid Excitotoxicity in Rat Magnocellular Nucleus Basalis. Effect of Cortical Deafferentation on Cerebral Blood Flow Regulation and Implications for Alzheimer's Disease

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β-Amyloid Excitotoxicity in Rat Magnocellular Nucleus Basalis

Effect of Cortical Deafferentation on Cerebral Blood Flow Regulation and Implications for Alzheimer's Disease

TIBOR HARKANY,[a,c] BOTOND PENKE,[b] AND PAUL G.M. LUITEN[a]

[a]*Department of Animal Physiology, University of Groningen, Haren, the Netherlands*

[b]*Department of Medical Chemistry, Szent-Györgyi Albert Medical University, Szeged, Hungary*

ABSTRACT: Alzheimer's disease is the most common type of dementia with a still largely unclear etiopathology. One of the factors that may directly contribute to the development and progression of the disorder is the abundant accumulation of β-amyloid peptides (Aβ) in senile plaques. In the present account we review coherent *in vivo* experimental evidence that Aβ infusion into the rat magnocellular nucleus basalis (MBN) induces abrupt and persistent behavioral dysfunctions, perturbations of sensory information processing, storage, and retrieval. These substantial behavioral changes are due to the loss of cholinergic neurons in the MBN and their ascending projections to the frontoparietal cortex. Both neuroanatomical and neurochemical observations pinpoint that infusion of Aβ into the rat basal forebrain significantly decreases choline-acetyltransferase and acetylcholinesterase activities and the population of—probably—M2 muscarinic acetylcholine receptors in the cerebral cortex. Neuropharmacological data indicate that Aβ toxicity is mediated by an excitotoxic cascade involving blockade of astroglial glutamate uptake, sustained activation of *N*-methyl-D-aspartate receptors and an overt intracellular Ca^{2+} influx. These changes are associated with increased nitric oxide synthase activity in cortical target areas that may directly lead to the generation of free radicals. Besides, as microvessels of the neocortex receive direct input from the MBN we assume that the loss of cholinergic innervation and hence that of tonic cholinergic vasoregulation ultimately leads to disturbances of vascular (endothelial) function and nutrient supply that may directly enhance neuronal vulnerability during aging and in Alzheimer's disease.

INTRODUCTION

One of the striking neuropathological hallmarks of Alzheimer's disease (AD) is the abundant accumulation of a 39–42 amino acid residue-containing peptide, termed β-amyloid protein (Aβ), in brain regions associated with memory formation.

[c]Address for correspondence: Dr. Tibor Harkany, Department of Animal Physiology, University of Groningen, Kerklaan 30, P.O. Box 14, NL-9750 AA Haren, the Netherlands. Tel.: +31 50 3632 353; fax: +31 50 3635 205.
e-mail: harkanyt@biol.rug.nl

Appearance of senile plaques with Aβ as their major constituent has long been regarded as a causal factor of AD. During the past decade a multitude of both *in vitro*[1–3] and *in vivo*[4–10,12–15] studies demonstrated the direct toxicity of Aβ or its derivatives, such as $A\beta_{25–35}$. Initially, Yankner *et al.*[1] demonstrated that Aβ is a neuroactive substance with a bimodal action profile. Whereas low (pM–nM) Aβ concentrations promote neural differentiation and growth, above a threshold level Aβ is toxic to nerve cells. As Aβ aggregates rapidly in aqueous solutions a critical role for the assembly state of the peptide was proposed to act as a pivotal determinant of its toxic effects.[2] Further, characterization of a molecular cascade mediating the neurotoxicity of Aβ on neurons has became a primary focus of *in vitro* studies and the involvement of Ca^{2+}-mediated excitotoxicity[3] and excess generation of free radicals emerged.[11]

Soon after the pioneering *in vitro* investigations on Aβ neurotoxicity Kowall *et al.*[12] reported toxic properties of Aβ in rat brain. During the past few years a broad spectrum of *in vivo* models were implicated in Aβ neurotoxicity research, each however with several limitations, like (I) substantially different concentrations and aggregation states of Aβ fragments, (II) a variety of peptide infusion protocols such as (i) acute local injections,[4–10,12,15] (ii) chronic Aβ infusion,[13] or (iii) intravascular perfusion of Aβ derivatives;[14] (III) differences of target brain areas (e.g., the hippocampus, the cerebral cortex, or basal forebrain nuclei), and (IV) different animal species.[15]

In the present account we report evidence for an excitotoxic nature of Aβ in an *in vivo* animal model of Aβ neurotoxicity which is based on amyloid peptide infusions in the magnocellular nucleus basalis (MBN). Furthermore, we will elaborate on the consequences of Aβ-induced cholinergic damage for cortical cerebral blood flow regulation and neuronal viability.

THE MAGNOCELLULAR NUCLEUS BASALIS MODEL OF Aβ NEUROTOXICITY: BASIC OBSERVATIONS

Cholinergic neurons of the MBN provide the majority of cholinergic input to neocortical structures, particularly to the more frontal and parietal cortical areas.[16] Ascending cholinergic projections from distinct sub-regions of the MBN exhibit a topologically defined unilateral cortical innervation pattern, such that cholinergic projection fibers originating in the intermediate MBN (iMBN) invade the frontoparietal somatosensory cortex.[16] Lesions to the iMBN have long been used to study the anatomical and functional consequences of neurotoxin infusion, such as that of kainic acid,[17] or *N*-methyl-D-aspatrate (NMDA).[18] Damage to the cholinergic neurons of the MBN elicits profound disturbances of both spontaneous animal behaviors and memory formation.[6,7]

To investigate any direct cholinotoxic effect of Aβ or Aβ-related peptides like $A\beta(Phe(SO_3H)^{24})25–35$, Aβ was infused into the iMBN in a 200 μM concentration (0.2 nmol/μl). Aβ-induced lesions in the iMBN resulted in a broad range of behavioral dysfunctions, such as pronounced anxiety in the elevated plus maze (FIG. 1A), immobility in the small "open-field" (FIG. 1B) and in an "open-field."[6] Loss of memory acquisition became apparent in a one-way step-through passive avoidance paradigm (FIG. 1C), but not in the Morris water maze (FIG. 1D). The latter observation

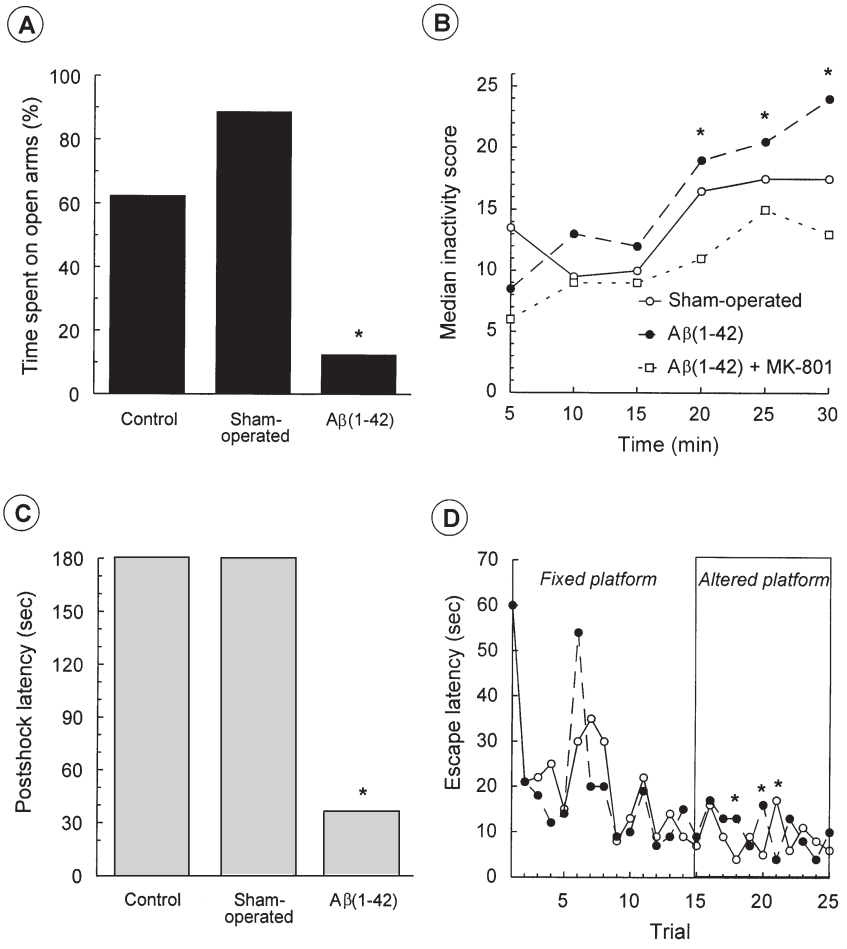
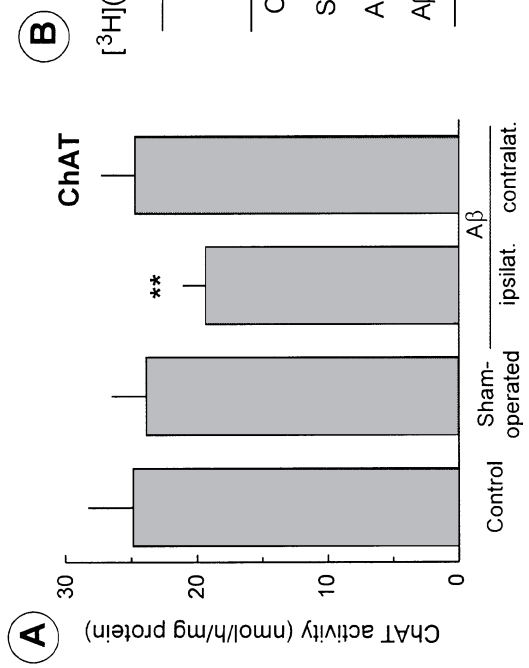


FIGURE 1. Behavioral consequences of $A\beta_{1–42}$ infusion into the rat MBN in four paradigms for the assessment of spontaneous behaviors (**A:** elevated plus maze, **B:** small "open-field") and learning and memory performance (**C:** one-way step-through passive avoidance, **D:** Morris water maze). Note the significant decrease of open arm entries in the elevated plus maze (A), the increased immobility (resting) frequency (B), and the loss of short-term memory in the postshock latency trial (24 h) of the passive avoidance test (C). Spatial learning capacity of $A\beta_{25–35}$-lesioned rats was not altered, relative to sham-operated animals (D). $A\beta_{1–42}$-induced behavioral dysfunctions were antagonized by acute pretreatment with the NMDA receptor blocker MK-801 (5 mg/kg of body weight, B). $^*p < 0.05$ (Mann-Whitney test); data are presented as medians.



B

[^{3}H](-)QNB binding parameters to rat frontal cortical membranes

	B_{max} (fmol/mg protein)	K_d (nM)
Control	504.57 ± 52.8	0.73 ± 0.2
Sham-operated	499.14 ± 153.5	0.68 ± 0.3
Aβ(1-42), ipsilateral	402.00 ± 94.8 *	0.58 ± 0.2
Aβ(1-42), contralateral	465.83 ± 121.7	0.76 ± 0.4

FIGURE 2. *Caption on following page.*

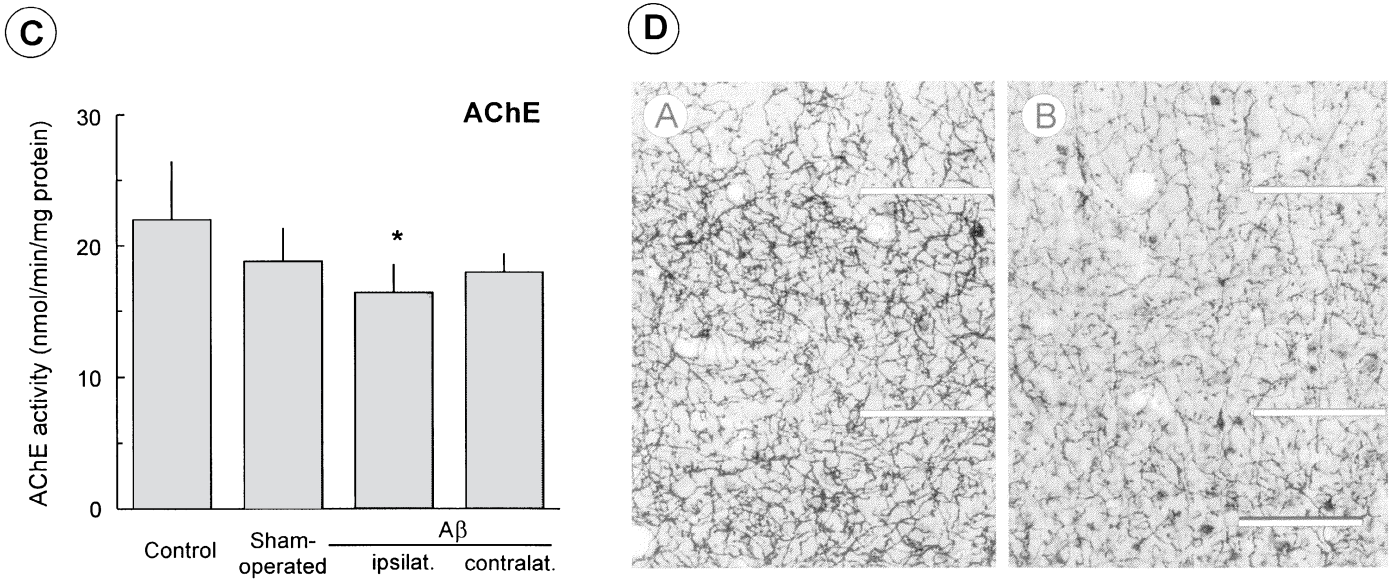


FIGURE 2. ChAT **(A)** and AChE **(C)** activities, and changes in receptor binding density (B_{max}) and dissociation constant (K_d) for [^{3}H](−)QNB binding in frontal cortical regions **(B)** of control, sham-operated and $A\beta_{1–42}$-treated animals. $A\beta_{1–42}$ injections into the MBN resulted in a significantly decreased ChAT activity (A) in the frontal cortices ipsilateral to the MBN injection as compared to all other groups examined, while AChE activity (C) in the ipsilateral frontal cortices was significantly reduced as compared to the control group. Note the extensive changes in the B_{max} value after $A\beta_{1–42}$ infusion into the MBN, while K_d remained unchanged. **(D)** Distribution of AChE-positive cortical cholinergic fibers originating in the damaged MBN subdivision. D/A represents a sham-lesioned, while D/B a $A\beta_{1–42}$-injected animal. $^{**}p < 0.01$, $^{*}p < 0.05$ (Student's *t*-test); data are reported as means ± SD. *Horizontal bars* in (D) depict layer V of the somatosensory cortex; *scale bar* = 150 μm.

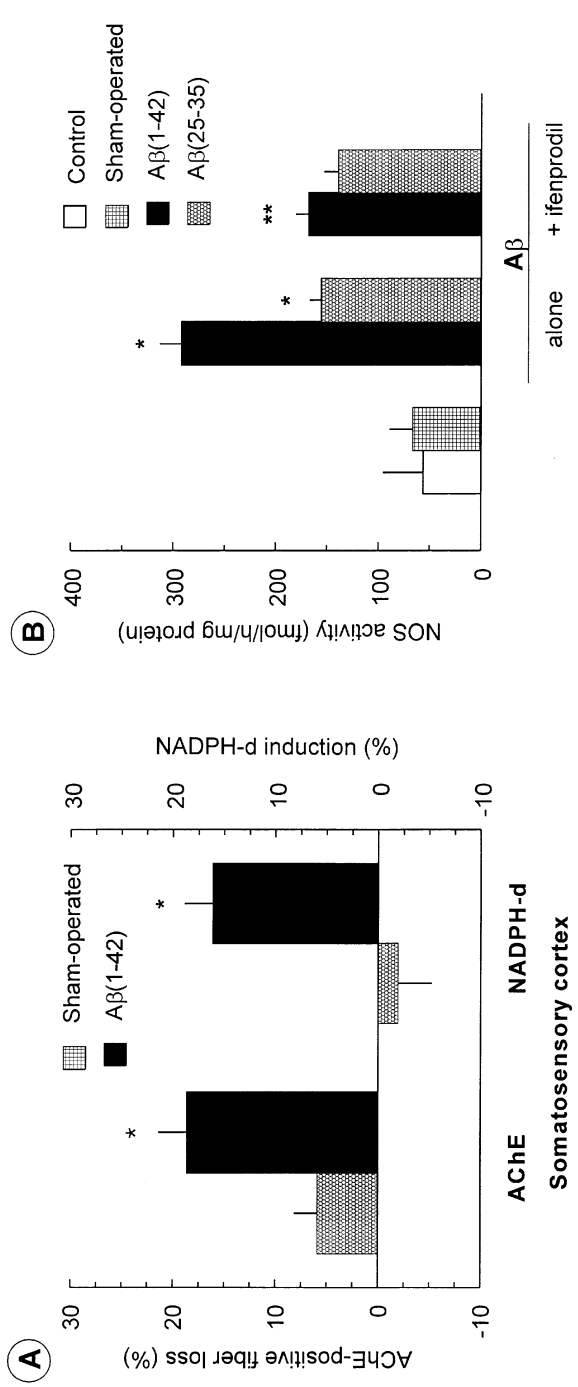


FIGURE 3. Loss of cholinergic (AChE-positive) projection fibers and activation of cortical NOS (**A**) 7 days postsurgery as a consequence of $A\beta_{1-42}$ infusion into the MBN. Injections of $A\beta_{1-42}$ elicited a significant loss of cholinergic projection fibers, whereas peptide infusion resulted in a marked activity increase of NOS as compared to sham-operated animals. (**B**) Cortical NOS activity and the effects of chronic ifenprodil (10 mg/kg of body weight, from 24 h postsurgery on) posttreatment following $A\beta_{1-42}$ or $A\beta_{25-35}$ infusions into the MBN. $^{*}p < 0.05$ vs. both naive and sham-control animals, while $^{**}p < 0.01$ vs. $A\beta_{1-42}$ (one-way analysis of variance). Data were expressed as percentages of the value of the contralateral side (A) and represent means ± SEM (A,B).

indicates target specificity of Aβ lesion, as hippocampus-related spatial learning performances were not altered. Neurochemical[4,7–9,19] and neuroanatomical[5,10,20] studies showed that detrimental effects of Aβ on cholinergic MBN function contribute to the behavioral disturbances. Altered cholinergic function involves significantly decreased choline-acetyltransferase (ChAT, FIG. 2A) and acetylcholinesterase (AChE, FIG. 2C,D) activities and the loss of M2, but not M1 muscarinic acetylcholine receptor (mAChR) binding sites (FIG. 2B[4]) in the fronto-parietal neocortex. These observations were further substantiated by histochemical experiments showing the loss of AChE-positive projection fibers to the somatosensory cortex (FIG. 2D).[6,8–10]

Aβ CHOLINOTOXICITY IS MEDIATED BY AN EXCITOTOXIC CASCADE

Whereas concentration- and amino acid sequence-dependent cholinotoxic properties of Aβ fragments could be firmly established, the identification of the cellular cascade by which Aβ elicits neurodegeneration is still largely unclear. Neuropharmacological data point to the involvement of a sustained NMDA receptor activation

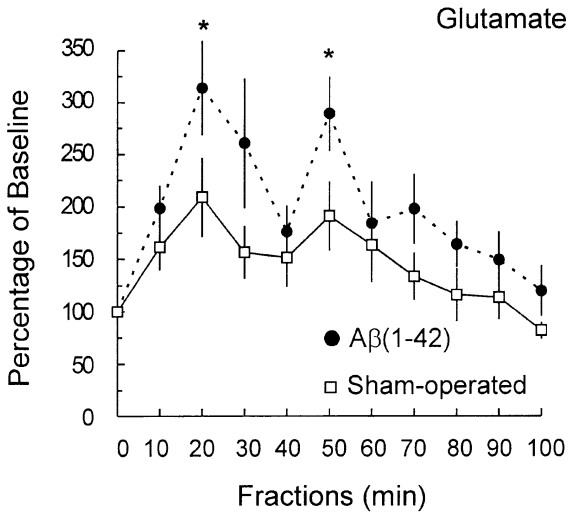


FIGURE 4. Time-profile of the extracellular glutamate concentration in the MBN of the rat following continuous microdialysis infusion of $A\beta_{1-42}$ in a 200 μM concentration. Extracellular concentration of glutamate exhibited rapid increases and reached its peak 20 min after the start of $A\beta_{1-42}$ infusion. Moreover, subsequent to the initial peak of the extracellular levels of glutamate, a second transient increase occurred at 50 min followed by a gradually decrementing profile of the extracellular amino acid content. $^*p < 0.05$ vs. sham-operated (one-way analysis of variance). Data are expressed as percentages of the baseline (means ± SEM).

in Aβ-induced pathological cellular signaling,[7–10] which is supportive to the leading hypothesis that an intracellular Ca^{2+} overload concomitant with an excess production of free radicals mediates Aβ toxicity on cultured neurons.[3,11] In this regard, both acute and chronic blockade of NMDA receptor function was proved to be highly effective in antagonizing Aβ toxicity *in vivo*[7,9,10] (FIG. 3B). Moreover, the fact that nitric oxide synthase (NOS) is activated in the neocortex upon infusion of Aβ into the MBN suggests a critical role for Ca^{2+}-triggered free radical generation (FIG. 3A). As such, vitamin E has recently been proven to be effective in the prevention of Aβ neurotoxicity,[7,21] which also points towards a role for free radicals in cascades mediating Aβ toxicity.

In vitro and *in vivo* data on Aβ neurotoxicity[9] together with the determination of excitotoxin-like properties of Aβ upon microdialysis infusion of the peptide into the MBN[8] revealed that Aβ initiates an excitotoxic cascade in the rat brain. Peptide infusion results (i) in the blockade of glutamate uptake by astrocytes that leads to (ii) a rapid and sustained increase of the extracellular concentrations of excitatory amino acid (EAA) neurotransmitters, such as aspartate and glutamate (FIG. 4). (iii) EAAs exert their effects by overt stimulation of their respective receptors including the NMDA receptor resulting in (iv) an intracellular Ca^{2+} ($[Ca^{2+}]_i$) overload. Sustained pathological concentrations of $[Ca^{2+}]_i$ may influence not only NOS activity, but also the function of intracellular Ca^{2+} pools, such as the mitochondria. Uncontrolled Ca^{2+} transients in mitochondria elicit uncoupling of the terminal oxidation cascade and lead to the generation of free radicals. Generally speaking, Ca^{2+}-mediated pathological Aβ signaling therefore ultimately leads to a subsequent formation of free radicals, and the two pathways converge to activation of "death signal" genes and fragmentation of DNA.

CHOLINERGIC DENERVATION OF CORTICAL MICROVESSELS: IMPLICATIONS FOR THE PATHOLOGY OF ALZHEIMER'S DISEASE

Besides the modulation of learning and memory formation in neocortical areas, cholinergic fibers originating in the basal forebrain influence vascular function and regional cerebral blood flow.[17] Ascending cholinergic fibers establish close but not direct contacts with endothelial cells of microvessels in the fronto-parietal neocortex.[22,23] Cholinergic end-feet were localized exclusively on mAChR-positive astroglial soma or leaflets (FIG. 5A–D) surrounding microvessels in a close (<30 μm) proximity to endothelial cells.[22,23] This feature enables direct regulation of regional blood flow and blood-brain barrier function if volume transmission of acetylcholine is considered.[17,22] These recent results indicate that interactions of the structural triads of ChAT-positive cholinergic perivascular terminals, mAChR-positive cholinoceptive astroglia, and endothelial cells are a key element in the neurogenic control of the intracortical microcirculation.

From a functional but also a pharmacological point of view cholinergic denervation profoundly influences local blood microcirculation, nutrient supply, and NOS activation.[10,17] Enhanced NO generation[10] can be postulated to modulate endothelial function in a bimodal manner. Increased NO production during the acute phase of Aβ-induced degeneration of cholinergic perivascular terminals results in transient

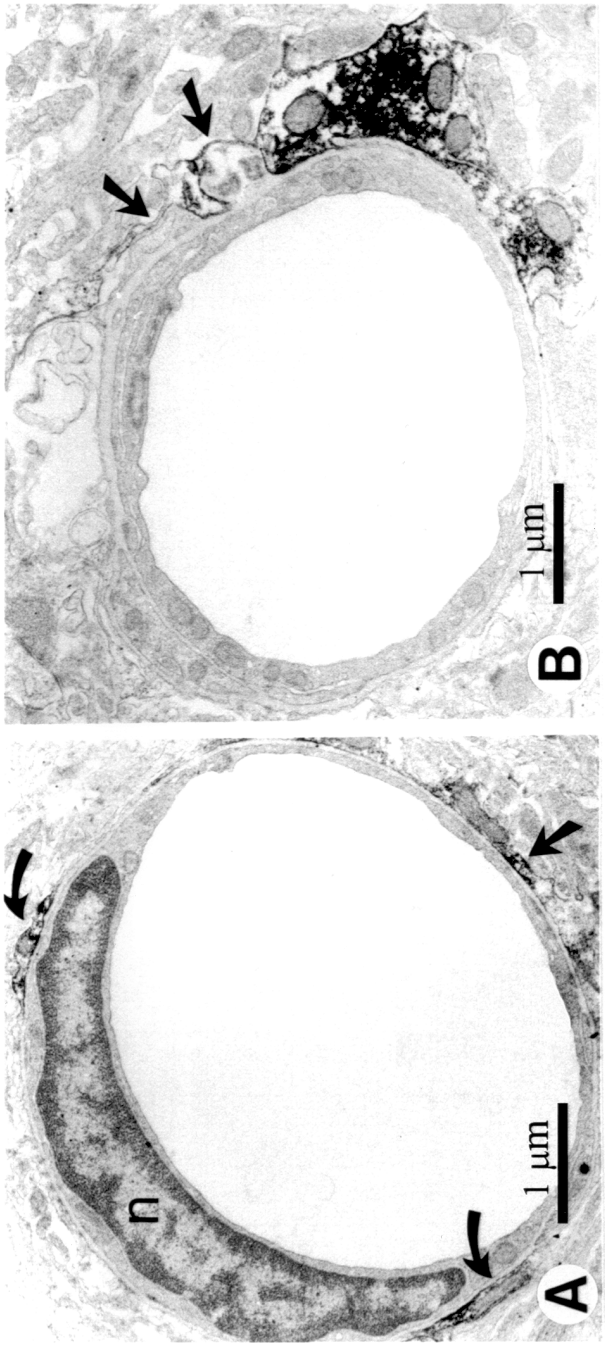


FIGURE 5. *Caption on following page.*

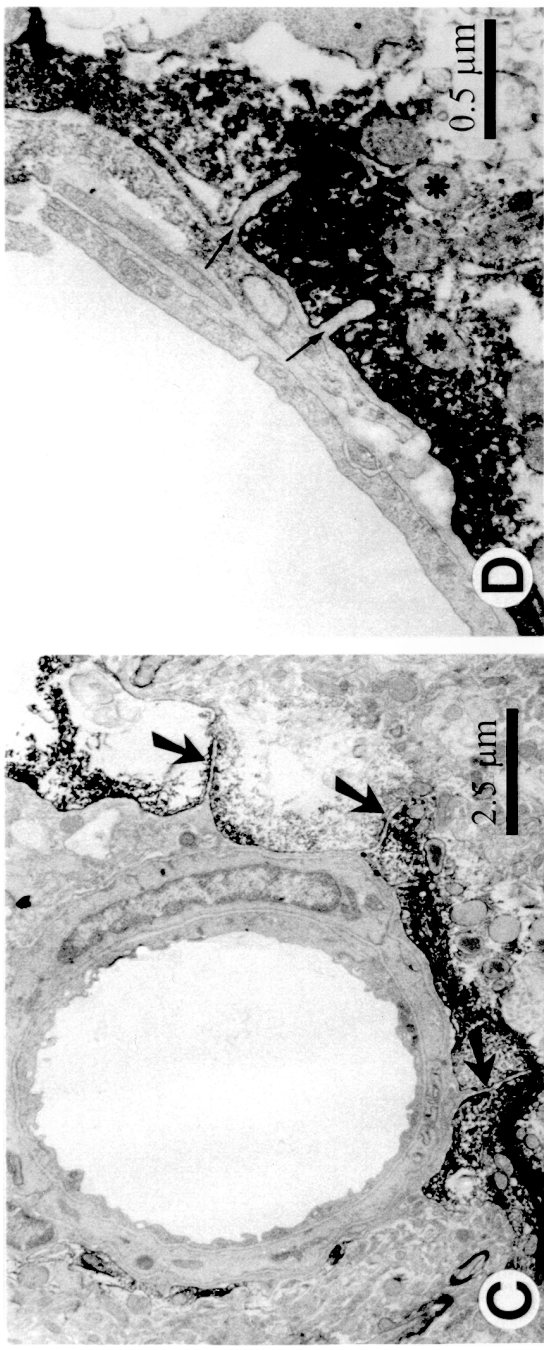


FIGURE 5. **(A)** Survey of a cortical capillary with a smooth and thin layer of cytoplasm in which several mitochondria and a large flattened nucleus can be identified. The endothelium is surrounded by a thin basement membrane. Several immunolabeled astrocytic processes are present in apposition to the basement membrane (*large arrows*). **(B)** Astrocyte immunoreactive for muscarinic receptor protein. A large, darkly labeled endfeet is connected by fine processes (*arrows*) to extensions protruding into the neuropil. **(C)** Large astrocytic complex with separate mAChR immunostained components in direct contact with the perivascular basement membrane. The compartments are separated by fingershape extensions of the basement membrane (*arrows*). **(D)** The basement membrane extensions as in (C) are indicated here at a higher magnification. Mitochondria are marked with an *asterisk*.

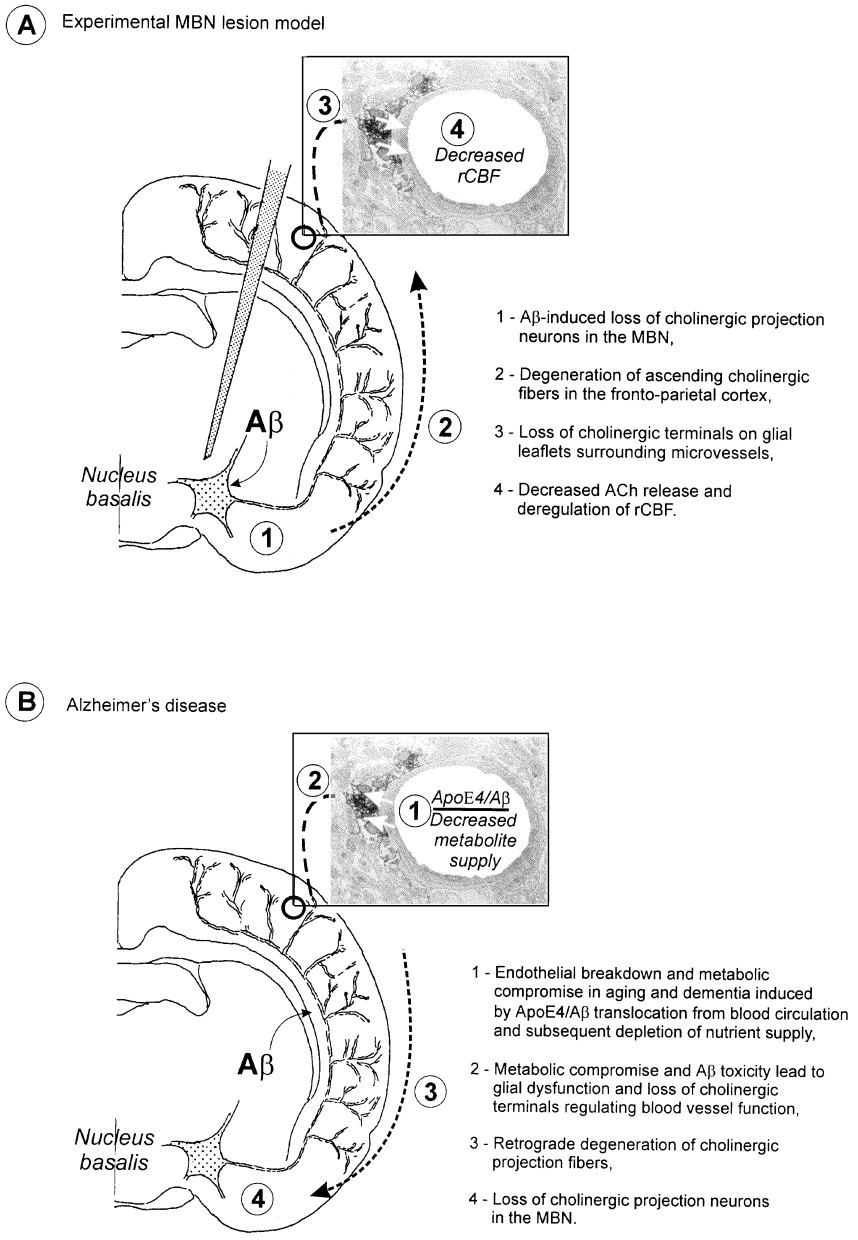


FIGURE 6. Comparison of successive steps of the neurotoxic cascades in the MBN lesion model of Aβ toxicity and during aging and Alzheimer's disease. Note the reversed sequence of events.

vasodilatation, whereas the permanent loss of cholinergic end-feet deregulates perivascular astroglial function and will lead to a reduced regional blood flow in the cerebral cortex. As aging is frequently associated with significantly decreased pO_2 and a metabolic compromise,[24] disturbances of cerebral blood flow regulation and endothelial function may render neurons vulnerable to excitotoxic (e.g., Aβ-induced) damage.

DISCUSSION

In conclusion, the data presented here provide compelling experimental evidence that Aβ and its derivatives exert cholinotoxicity[5] in the rat MBN. The neurotoxic action of Aβ is mediated by an excitotoxic cascade involving sustained activation of NMDA receptors and subsequent induction of NOS and generation of free radicals. Aβ-induced lesions to the MBN may deregulate cerebral blood flow and blood-brain barrier characteristics via the perturbation of both NO- and acetylcholine-mediated control of microvascular function. It is worth noting that exposure of MBN cells to Aβ induces a neurotoxic cascade that may affect cholinergic neuron integrity during aging and in AD in two ways (FIG. 6). First, Aβ triggers damage to cholinergic projection neurons, and second, this cholinergic neuron injury is the primary signal in the MBN reducing vasomotility and cerebral blood flow. As a result, such a chronic condition of hypoperfusion and impaired nutrient supply accompanied by the translocation of apolipoprotein E4/Aβ complexes from the blood circulation to brain parenchyma[25] will be a threat to intracortical cholinergic nerve terminals and by retrograde mechanisms can accelerate degeneration of cholinergic fibers and an ultimate loss of cholinergic MBN neurons in Alzheimer's disease.

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